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Synthesis and post-translational assembly of intermediate filaments in avian erythroid cells: Vimentin assembly limits the rate of synemin assembly

(immunoprecipitation/phosphorylation/protein assembly/cytoskeleton)

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ABSTRACT **The assembly of vimentin intermediate filaments and the high molecular weight filament crosslinking protein, synemin, was studied in erythroid cells from 10-day chicken embryos. Pulse labeling studies show that newly synthesized vimentin is present both in a Triton X-100-insoluble form and in a soluble form. The incorporation of labeled vimentin into the insoluble fraction increases linearly with time, while the soluble pool of labeled vimentin saturates quickly. In contrast, synemin accumulates rapidly in the Triton X-100-soluble fraction and begins to accumulate in the insoluble fraction only after a considerable lag of time. Pulse–chase studies reveal that the detergent-soluble pools of both vimentin and synemin contain precursors for their post-translational assembly into detergent-insoluble filaments and that the half-life of soluble synemin is about twice as long as that of soluble vimentin. Immunoprecipitation of solubilized filaments with synemin antiserum precipitates vimentin with synemin. On the other hand, soluble vimentin does not coimmunoprecipitate with soluble synemin. These results suggest that, in the assembly of vimentin and synemin into intermediate filaments, vimentin filament elongation generates synemin binding sites, and thus the rate of vimentin filament elongation limits the rate of synemin assembly.**

The cytoplasm of higher eukaryotic cells is populated by three major classes of filaments: actin filaments, microtubules, and intermediate filaments. Each filament class is a multipolypeptide assembly composed of one or two major subunits that form the core of the polymer and a number of accessory polypeptides whose role is to modulate the structure and function of the filaments during mitosis and cell movement, or during development and differentiation (for reviews see refs. 1–3). Even though the association of a number of accessory proteins with their core subunits has been studied extensively *in vitro*, the regulation of the assembly of these accessory proteins with the core subunits *in vivo* has not been adequately investigated.

To approach this issue we have chosen the intermediate filament network in chicken erythrocytes as a model system. Chicken erythrocytes are nucleated, unlike mammalian erythrocytes, and contain a plasma membrane-associated spectrin–actin network (4). They also contain a marginal band of microtubules and a system of filaments that interlinks the nucleus and the plasma membrane. These filaments have been identified recently as intermediate filaments (for review see ref. 5). Immunological and biochemical analyses of these intermediate filaments have shown that they have a relatively simple composition. The core component is the 52,000-dalton polypeptide vimentin. Associated with the vimentin filaments is a 230,000-dalton polypeptide, synemin (5), initially found in association with the muscle intermediate filament subunit proteins, desmin and vimentin (6). In erythrocytes synemin is about 2% as abundant as vimentin on a molar basis (5), binds peripherally to the vimentin filaments at periodic intervals of 180 nm in adult erythrocytes (230 nm in erythroid cells from embryos), and functions to crosslink them (2).

Because synemin is peripherally and periodically associated with the filaments, how is its association with elongating vimentin filaments regulated? We have followed the kinetics of assembly of synemin with respect to those of vimentin and have found that newly synthesized synemin, similar to vimentin (7), enters a detergent-soluble pool before being assembled onto the filaments. However, the assembly of vimentin and synemin into the growing filaments appears to be noncoordinate due to the longer half-life in the soluble pool of synemin relative to vimentin. This suggests that the elongation of vimentin filaments limits the assembly of synemin.

MATERIALS AND METHODS

Cell Preparation, Labeling, and Lysis. Erythroid cells were isolated from 11-day chicken embryos, filtered, and washed as described (7). Washed cells were suspended to 10% (vol/vol) in minimal essential medium depleted of either methionine or phosphate and containing 10% dialyzed calf serum. Suspensions of cells (1.5 ml, 5×10^8 cells per ml) were then labeled continuously for 90 min at 35°C with 1.3 mCi (1 Ci = 3.7×10^{10} Bq) of [^{35}S]methionine (1,250 Ci/mmol, Amersham) or 10 mCi of $H_3{}^{32}PO_4$ (carrier-free; New England Nuclear). Pulse–chase experiments were carried out simultaneously by labeling cells with [^{35}S]methionine for 5 min, followed by the addition of unlabeled methionine to a final concentration of 1.6 mM.

To monitor the synthesis, phosphorylation, and incorporation into the cytoskeleton of vimentin and synemin, 250-μl aliquots of labeled cells were withdrawn at the times indicated in the figure legends, diluted into 15 ml of 155 mM choline chloride/5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4, at 0°C and pelleted in a clinical centrifuge at $500 \times g$ for 4 min. The supernatant was removed and the cells were lysed by suspension in 10 vol of lysis buffer [100 mM KCl/300 mM sucrose/20 mM 1,4-piperazinediethanesulfonic acid (Pipes)/1 mM $Mg(OAc)_2$/5 mM ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA)/0.5% Triton X-100, pH 6.8]. After 2.5 min at 0°C, the cytoskeletons were pelleted at $12,500 \times g$ for 5 min in 1.5-ml conical microcentrifuge tubes. The supernatant, consisting of the detergent-soluble fraction, was transferred to new centrifuge tubes and the detergent-insoluble cytoskeletal fraction was suspended in lysis buffer to the same volume as the soluble fraction. Urea and 2-mercaptoethanol were then added to final concentrations of 9 M and 2.5% (wt/vol), respectively.

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Immunoprecipitation and Gel Electrophoresis. Antibodies directed against chicken embryonic skeletal muscle vimentin and chicken gizzard synemin were those described previously (6, 8). Aliquots of the [^{35}S]methionine or $H_3{}^{32}PO_4$-labeled soluble and cytoskeletal samples were processed for immunoprecipitation by using method B described previously (9), followed by separation of the immunoprecipitated polypeptides on $NaDodSO_4$/10% polyacrylamide slab gels or two-dimensional isoelectric focusing $NaDodSO_4$/polyacrylamide gels (6). Gels were fluorographed (7) by using preflashed Kodak XAR-5 x-ray film (10).

RESULTS

Soluble and Cytoskeletal Distribution of Newly Synthesized Vimentin and Synemin. Fig. 1 shows that, in contrast to the cytoskeletal distribution of vimentin (7) and synemin (Fig. 1*E*) at steady state, newly synthesized vimentin and synemin are present in both the soluble and cytoskeletal fractions during a 90-min labeling period with [^{35}S]methionine (Fig. 1 *A* and *B*). Quantification of these data (Fig. 2) demonstrates that during the first 15 min of labeling newly synthesized vimentin is found primarily in the soluble fraction (Fig. 1*A*, lane 2 vs 8, and Fig. 2*A*). The amount of newly synthesized vimentin in the soluble fraction continues to increase until 30 min, after which time the amount of labeled vimentin saturates the soluble pool, as evidenced by a plateau in the amount of [^{35}S]methionine-labeled soluble vimentin (see also ref. 7). Newly synthesized vimentin is found in the cytoskeleton after a 15-min incubation in the presence of [^{35}S]methionine but in lesser amounts than are present in the soluble fraction. The incorporation of newly synthesized vimentin into the cytoskeleton then continues in a linear fashion during the 90-min incubation period. After 45 min of labeling, the amount of labeled vimentin in the cytoskeleton has exceeded the amount in the soluble fraction.

Similar to vimentin, the amount of newly synthesized synemin in the soluble fraction exceeds the amount in the cytoskeleton for the first 45 min of labeling (Figs. 1*B* and 2*B*). However, in contrast to vimentin, the amount of labeled synemin in the soluble fraction does not plateau at this time but continues to increase. The amount of newly synthesized synemin in the soluble fraction does eventually plateau like vimentin, albeit at a later time. This is demonstrated by the pres-

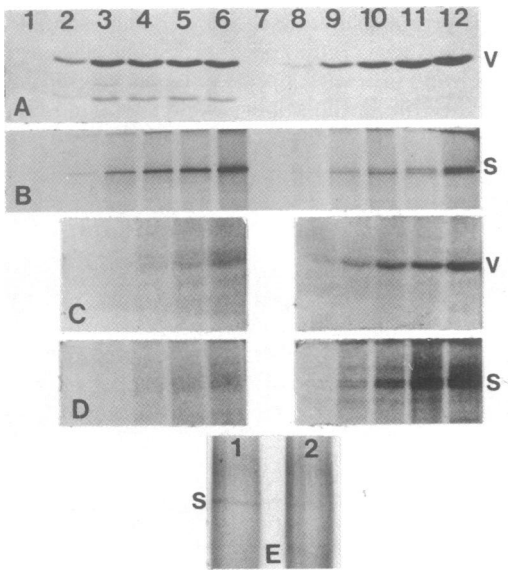

FIG. 1. Immunoprecipitation of labeled vimentin and synemin from soluble and cytoskeletal fractions of erythroid cells. Erythroid cells from 11-day embryos were labeled with [^{35}S]methionine (*A* and *B*) or $^{32}PO_4$ (*C* and *D*) for various lengths of time that yield linear total [^{35}S]methionine incorporation (data not shown), then separated into Triton X-100-soluble fractions (lanes 1–6) and cytoskeletal fractions (lanes 7–12) before immunoprecipitation of vimentin (V) and synemin (S), followed by gel electrophoresis and fluorography. Labeling times in *A-D* were 5 min (lanes 1 and 7), 15 min (lanes 2 and 8), 30 min (lanes 3 and 9), 45 min (lanes 4 and 10), 60 min (lanes 5 and 11), and 90 min (lanes 6 and 12). A Coomassie blue-stained gel (*E*) of immunoprecipitates shows synemin is predominantly in the cytoskeletal fraction (lane 1), with very little in the soluble fraction (lane 2), in 11-day chicken embryo erythroid cells. When soluble and cytoskeletal synemin are electrophoresed in adjacent lanes, the soluble synemin migrates with the upper component of the cytoskeletal synemin doublet (data not shown). Because the lower component of this doublet is not obtained consistently (see Fig. 4, lane 4), it was omitted from quantification. Whether the lower component is related to synemin or represents immunoprecipitated β-spectrin (220,000 daltons) is presently unknown. Gels were exposed to x-ray film for 5.5 hr (*A*), 18 hr (*B*), and 4 days (*C* and *D*). These data are quantified in Fig. 2.

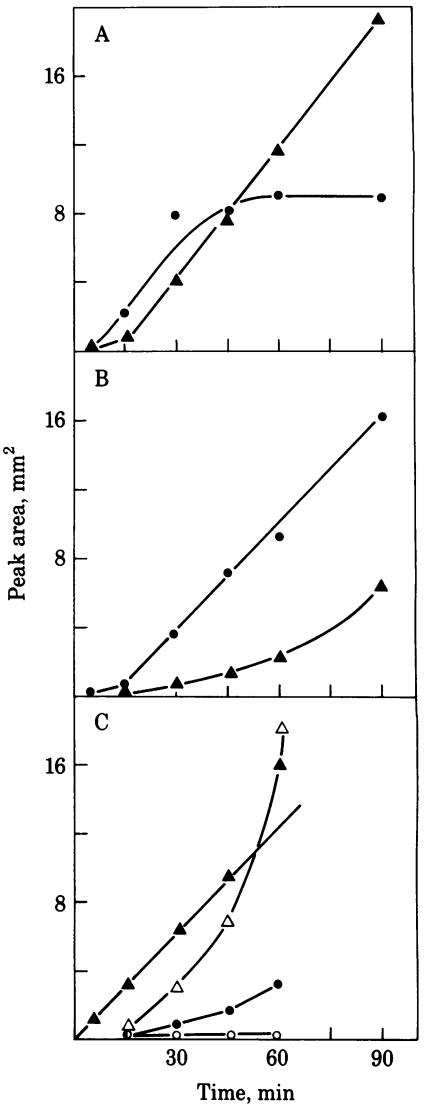

FIG. 2. Kinetics of synthesis, assembly, and phosphorylation of vimentin and synemin in chicken embryo erythroid cells. Fluorographs from Fig. 1 of immunoprecipitated vimentin and synemin were scanned with a densitometer and the peak areas are plotted. Kinetics are of [^{35}S]methionine-labeled vimentin (*A*) and synemin (*B*) in the soluble (●) and cytoskeletal (▲) fractions. (*C*) $^{32}PO_4$-Labeled vimentin in the soluble (●) and cytoskeletal (▲) fractions, and synemin in the soluble (○) and cytoskeletal (△) fractions.

ence in the cytoskeleton of most of the synemin at steady state (Fig. 1E) and by separate experiments in which labeled synemin in the soluble pool reached a plateau within 90 min (data not shown). Newly synthesized [^{35}S]methionine-labeled synemin enters the cytoskeleton at a slower initial rate than vimentin and in a nonlinear fashion (Fig. 2B). As described later, these nonlinear kinetics are attributed to the slowly increasing specific activity of the soluble pool of synemin polypeptides, which serve as precursors for the synemin incorporated into the vimentin filaments.

Quantification of the amount of newly synthesized vimentin and synemin in both the soluble and cytoskeletal fractions reveals that about 20-fold more vimentin than synemin is synthesized in erythroid cells from 11-day chicken embryos. This approximation was determined by correcting densitometer tracings of the fluorographs for the approximately seven methionine residues in mammalian vimentin (11) and the 23 methionine residues in chicken synemin (12). While the 20:1 ratio of synthesis of these polypeptides differs from the estimated steady-state ratio of 50:1 in mature erythrocytes (5), it is possible that the 50:1 steady-state ratio is biased by nonlinearity in staining by Coomassie blue (5). Alternatively, the 20:1 ratio of synthesis may itself obscure differential turnover or changes in the rates of synthesis of these polypeptides during development.

Phosphorylation of Vimentin and Synemin Occurs in the Cytoskeletal Fraction. Both vimentin and synemin are phosphorylated in chicken embryo erythroid cells (Figs. 1 and 2). However, unlike the partitioning of [^{35}S]methionine-labeled polypeptides into both the soluble and cytoskeletal fractions, the phosphorylated vimentin and synemin are found primarily in the cytoskeletal fraction (Figs. 1 C and D, and 2C). The time course of phosphorylation of synemin in the cytoskeleton is similar to that of vimentin in the same fraction (Fig. 2C).

Soluble Vimentin and Synemin Serve as Precursors for Insoluble Filaments. The presence of newly synthesized vimentin and synemin in both the soluble and cytoskeletal fractions raises the possibility that the soluble polypeptides serve as precursors for the post-translational assembly of insoluble intermediate filaments. To elucidate this potential relationship, erythroid cells were pulsed with [^{35}S]methionine for 5 min, and then the labeled polypeptides were chased with unlabeled methionine for 90 min. As shown in Fig. 3A and previously (7), upon commencement of the chase, the amount of labeled vimentin in the cytoskeletal fraction increases rapidly while the amount of labeled vimentin in the soluble fraction decreases. Because the total amount of labeled vimentin remains constant, the shift in distribution of vimentin from the soluble to the cytoskeletal fraction indicates that soluble vimentin serves as a precursor for the cytoskeletal vimentin with little or no detectable turnover (see also ref. 7).

The same samples were analyzed for the distribution of synemin (Fig. 3B). When the total amount of [^{35}S]methionine recovered in full-sized synemin has reached a plateau (30–45 min, Fig. 3B), the amount of synemin in the cytoskeleton increases, while the amount in the soluble fraction decreases. Thus, the soluble pools of both vimentin and synemin serve as precursors for the vimentin and synemin present in the cytoskeleton. The data do not exclude the possibility that some soluble synemin is catabolized rather than assembled, though in the time scale of these experiments there is little detectable turnover. By estimating the time required for the [^{35}S]methionine in the soluble pool to decrease by 50%, we estimate that vimentin has a half-life of about 25 min in the soluble pool (see ref. 7 for calculations), while synemin has a half-life of about 65 min. This suggests that there is a larger soluble pool of synemin

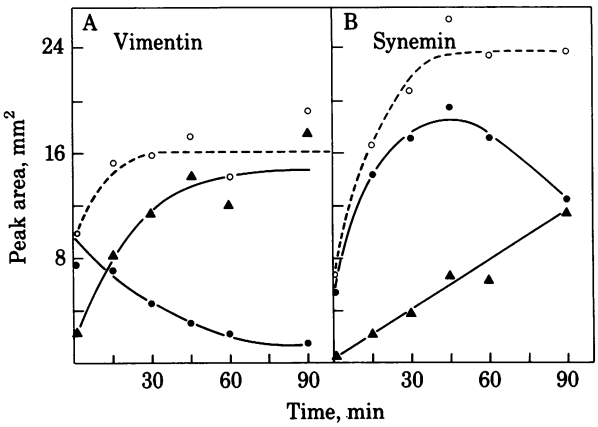

FIG. 3. Post-translational assembly of vimentin (A) and synemin (B) from Triton X-100-soluble pools. Chicken embryo erythroid cells were pulsed with [^{35}S]methionine for 5 min, then the methionine pool was diluted with unlabeled methionine. At various times during the incubation at 35°C, aliquots of cells were separated into soluble and cytoskeletal fractions and were processed for immunoprecipitation, gel electrophoresis, fluorography, and densitometry. The peak areas were then plotted to illustrate that the soluble (●) vimentin (A) and synemin (B) serve as precursors to cytoskeletal (▲) vimentin and synemin. The total amount of labeled vimentin and synemin (○) was determined by adding the amounts in the soluble and cytoskeletal fractions.

relative to its cytoskeletal binding sites.

From Fig. 3B it is evident that the total amount of labeled synemin in the erythroid cells continues to increase for 30–45 min after initiation of the chase with unlabeled methionine. Even though one would expect two transit times [6-min transit time, assuming elongation at five amino acids per sec (13)] after commencement of the chase for all labeled synemin nascent chains to be completed, the 30–45 min required to reach a plateau in labeled synemin seems rather long. However, vimentin requires about 15 min to reach a plateau in [^{35}S]methionine incorporation (Fig. 3A), which is a roughly proportional period of time given that it is one-fourth the size of synemin.

Association of Synemin with Vimentin. We have used an approach different from that used previously (2, 6) to examine the association of synemin with vimentin before and after assembly into filaments. After labeling of erythroid cells with [^{35}S]methionine and immunoprecipitation of the soluble and cytoskeletal fractions with anti-vimentin antibodies, we detected labeled material with a molecular weight of approximately 230,000 in addition to vimentin (Fig. 4A, lanes 1 and 2). Because this polypeptide may have represented either tetramers of vimentin or monomers of synemin, we immunoprecipitated labeled synemin from the same samples to look for coprecipitation of vimentin. Because immunoautoradiography (6) demonstrates monospecificity of the antibodies for synemin, we can rule out the presence of crossreactive antigenic determinants in the two proteins. Very little vimentin was coprecipitated from the soluble fractions with anti-synemin (Fig. 4A, lane 3), and in other experiments none was detected even though immunoprecipitation with vimentin antibodies shows that there is an abundance of labeled vimentin in the soluble fraction (Fig. 4A, lane 1). Strikingly, however, immunoprecipitation of synemin from the cytoskeletal fraction leads to coprecipitation of a labeled polypeptide that migrates with vimentin on NaDodSO$_4$/10% polyacrylamide gels (Fig. 4A, lane 4). Two-dimensional gel analysis of such immunoprecipitates indeed shows the presence of two polypeptides with isoelectric points corresponding to those of synemin and vimentin (Fig. 4B). To further investigate whether this polypeptide that immunoprecipitated with synemin was indeed vimentin and whether it was primarily

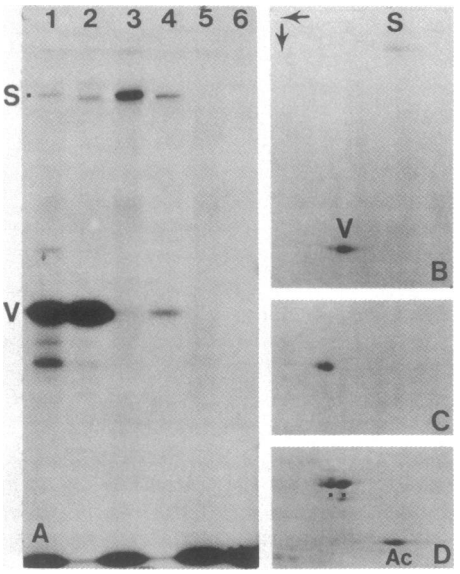

FIG. 4. Coimmunoprecipitation of vimentin and synemin from cytoskeletal fractions of erythroid cells. Erythroid cells from 10-day chicken embryos were labeled for 1 hr (35°C) with [^{35}S]methionine, separated into soluble and cytoskeletal fractions, and processed for immunoprecipitation with either vimentin or synemin antibodies, gel electrophoresis, and fluorography. (*A*) Immunoprecipitation with anti-vimentin serum of the soluble (lane 1) and cytoskeletal (lane 2) fraction and with anti-synemin serum of the same soluble (lane 3) and cytoskeletal (lane 4) fractions leads to recovery of vimentin (V) and synemin (S). Immunoprecipitation of soluble (lane 5) and cytoskeletal (lane 6) fractions with preimmune serum for the synemin serum, and vimentin serum (not shown) does not lead to the recovery of labeled polypeptides. Some of the labeled soluble material in lanes 1 and 2 that migrates similarly to synemin in lanes 3 and 4 is likely to represent aggregates of vimentin (see text). (*B* and *C*) In a separate experiment, isoelectric focusing (acidic end to the left), NaDodSO$_4$/10% polyacrylamide gel electrophoresis, and fluorography were used to monitor anti-synemin serum immunoprecipitates of [^{35}S]methionine- (*B*) and $^{32}PO_4$- (*C*) labeled cytoskeletal fractions. (*D*) Coomassie blue stain of the gel used for fluorography in *B*. In this experiment, total unlabeled cytoskeletons were mixed with the labeled immunoprecipitate to demonstrate that the labeled material (*B*) that coimmunoprecipitates with synemin when anti-synemin serum is used is primarily the basic nonphosphorylated form of vimentin (right-hand dot). The acidic Coomassie blue-stained variant of vimentin migrates with the $^{32}PO_4$-labeled vimentin in *C*. The presence of actin (Ac) in the Coomassie blue-stained gel is noted. Fluorographs were exposed for 4 days (*A*) and 2 days (*B* and *C*).

phosphorylated or nonphosphorylated vimentin, we mixed [^{35}S]-methionine- or $^{32}PO_4$-labeled immunoprecipitates with total cytoskeletons from chicken erythrocytes to allow visualization of vimentin by Coomassie blue staining. After two-dimensional gel electrophoresis, gel staining, and fluorography, the labeled polypeptide that precipitated with synemin when anti-synemin antibodies were used was identified as primarily nonphosphorylated vimentin by the direct correspondence of the [^{35}S]-methionine-labeled gel spot with the basic component of the Coomassie blue-stained vimentin (Fig. 4 *B* and *D*). Immunoprecipitation of $^{32}PO_4$-labeled cytoskeletal fractions revealed that at least some of the vimentin that precipitated with synemin is phosphorylated (Fig. 4 *C* and *D*).

DISCUSSION

Previous studies from this laboratory have established that the intermediate filament network of adult chicken erythrocytes is composed of vimentin as the core polypeptide and of synemin as a peripherally associated and periodically spaced polypeptide (2, 5). Furthermore, similar to intermediate filaments in other cells, erythrocyte intermediate filaments are insoluble in physiological buffers and in buffers containing nonionic detergents (5). The insolubility of the filaments raises the question of how cells synthesize and assemble these structures. One possibility is that assembly occurs cotranslationally as the nascent chains of these two polypeptides attain a certain length on the polyribosomes (14, 15). Alternatively, assembly may occur post-translationally and utilize soluble precursors. Previous work from this laboratory (7) demonstrated that newly synthesized vimentin enters a detergent-soluble pool before assembly into filaments, thus clearly establishing that, at least in the case of vimentin, assembly occurs post-translationally. In this study we have used the same approach to investigate the assembly kinetics of synemin with respect to those of vimentin. By using immunoprecipitation of the two polypeptides with synemin- and vimentin-specific antibodies, respectively, we could simultaneously follow the kinetics of synthesis and assembly of both polypeptides in the same cell population and under the same experimental conditions. We found that the assembly of synemin occurs post-translationally, similarly to that of vimentin. This is evident from the rapid accumulation of synemin in the soluble pool and the rather slow kinetics of accumulation of synemin in the insoluble filaments, and from data suggesting that synemin in the soluble pool can be chased into the filaments.

The distinctly different kinetics of accumulation of vimentin and synemin in the soluble and cytoskeletal fractions has implications for the regulation of assembly of these components. Initially, the accumulation of labeled vimentin in the soluble fraction is linear, and after 20–30 min the amount of soluble labeled vimentin reaches a plateau. At this plateau the number of molecules entering the soluble pool equals the number of molecules being incorporated into the filaments. The rapid saturation of the soluble pool suggests that the amount of soluble vimentin is not in great excess of cytoskeletal binding sites, and hence newly synthesized vimentin is rapidly incorporated into the cytoskeleton. On the other hand, synemin accumulation in the soluble fraction is linear for at least 90 min, and synemin begins to accumulate in the cytoskeletal fraction after a considerable lag of time. Because synemin is approximately four times larger in molecular weight than vimentin, this difference may in part reflect the fact that it takes synemin four times longer than vimentin to be synthesized and to saturate its soluble pool. The slower accumulation of synemin in the cytoskeletal fraction, compared with vimentin, may also reflect an initial dilution of radioactive synemin with nonradioactive synemin in a soluble pool that is larger, relative to the availability of its binding sites, than that of vimentin. The larger size of the synemin soluble pool is also suggested by the long half-life of soluble synemin, as determined by the pulse–chase experiment (Fig. 3). This observation, taken with evidence that synemin does not readily dissociate from filaments (see below), suggests that the rate of vimentin assembly limits the rate of synemin assembly onto the filaments.

Vimentin assembly may limit synemin assembly if the polymerization of vimentin generates synemin binding sites through some long-range suprastructure of the filament. This hypothesis would be consistent with the data presented here, which indicate that synemin preferentially associates with vimentin that already has been incorporated into the filaments rather than with soluble vimentin. The preferential association of synemin with cytoskeletal vimentin is implied by the observation that newly synthesized synemin is immunoprecipitated with newly synthesized vimentin by anti-synemin antibodies from the cytoskeletal fraction, but it is not immunoprecipitated with vimentin from the soluble fraction even though both polypep-

tides are present in this fraction.

The complicated kinetics of synemin synthesis and assembly raise the possibility that cytoskeletal synemin exchanges with soluble synemin. We consider this unlikely for the following reasons. First, previous studies have indicated that the association of synemin with intermediate filaments is strong, because synemin and desmin copurify through repeated cycles of depolymerization and repolymerization (6, 12) and can be dissociated only in the presence of urea at high concentrations (12). Second, our pulse–chase experiments suggest the unidirectional flow of soluble synemin to cytoskeletal synemin and not the establishment of an equilibrium with the cytoskeleton. Third, although newly synthesized [^{35}S]methionine-labeled synemin is about evenly distributed between the soluble and cytoskeletal fractions after short labeling times, $^{32}PO_4$-labeled synemin is present almost exclusively in the cytoskeletal fraction. Barring the preferential dephosphorylation of soluble synemin, this argues strongly against the release of cytoskeletal synemin into the soluble fraction.

On the basis of the above discussion we propose the following model for the assembly of vimentin and synemin into erythroid cell intermediate filaments. Newly synthesized vimentin first enters a small soluble pool. When in the soluble phase, vimentin may remain monomeric, rapidly form protofilaments, or perhaps form an oligomeric complex with proteins other than synemin. The soluble vimentin is then rapidly assembled into elongating detergent-insoluble intermediate filaments, and the rate of this conversion limits the rate of filament growth. It is not known how the size of the soluble vimentin pool influences assembly kinetics. Newly synthesized synemin also enters a soluble pool, but this pool initially exceeds available filament binding sites. We propose that the slow accumulation of synemin into the cytoskeleton from the soluble pool is indicative of a post-translational binding of synemin to vimentin filaments. This association may require the generation of synemin binding sites as a result of vimentin filament elongation and a higher-order change in vimentin structure. At some point during or subsequent to assembly, synemin and vimentin become phosphorylated. Finally, because the data from the pulse–chase experiment (Fig. 3) suggest that much of the soluble vimentin and synemin become incorporated into filaments during these short incubation times, it is likely that transcriptional or translational control, rather than proteolysis, are most important in determining the sizes of the soluble pools. These data do not preclude the possibility, however, that some of the soluble precursors are susceptible to catabolic processes and hence not incorporated into filaments. Although the results presented here and previously (7) are consistent with this model, alternative explanations are also possible, and further experimentation is necessary to test the validity of this model.

The validity of the above model of vimentin and synemin assembly is dependent upon the experimental approach of using operationally defined detergent-soluble and detergent-insoluble fractions. That these fractions reflect the partitioning of proteins into soluble and insoluble compartments within the cell is also suggested by data showing that soluble forms of newly synthesized vimentin (7) and spectrin (9) are obtained by lysing cells in a hypotonic buffer. It does, however, remain possible that detergent-unstable intermediates in the assembly of vimentin or synemin may be associated with the cytoskeleton and yet enter the soluble fraction during extraction with detergent.

The observation that both synemin and vimentin assemble post-translationally from a soluble precursor into an insoluble intermediate filament network has important implications for the assembly of cytoskeletal elements in general. Post-translational assembly does not appear to be limited to these two intermediate filament proteins, because we have also observed that the α and β subunits of spectrin, a membrane–skeletal protein in chicken erythroid cells, also appear to assemble post-translationally from a soluble precursor (9). Even though polyribosomes in general (16–21) and polyribosomes for α-spectrin in particular (22) are associated with the detergent-insoluble cytoskeleton, α-spectrin nascent chains are released from the cytoskeleton by puromycin (22). This further suggests that spectrin assembly occurs post-translationally. It is, therefore, possible that polyribosomes for many cytoskeletal components are associated with the cytoskeleton and perhaps segregated in the cell, but that the polyribosomes release completed polypeptides into a soluble pool prior to assembly. Our demonstration of the post-translational assembly of specific cytoskeletal polypeptides in chicken embryo erythroid cells yields a distinctly different view of cytoskeletal assembly than that obtained by examining large populations of unspecified cytoskeletal polypeptides in HeLa cells and 3T3 cells (14, 15). It is not known whether cytoskeletal assembly proceeds by different mechanisms in different cell types or if it is subject to regulation during development. It is, however, evident from our work that known cytoskeletal components such as vimentin, synemin, and spectrin are assembled only post-translationally in chicken embryo erythroid cells.

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